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(54) Title: ISOLATED NUCLEIC ACID MOLECULES WHICH ARE MEMBERS OF THE MAGE-B FAMILY AND USES THEREOF

(57) Abstract

The invention relates to members of the MAGE-B family of nucleic acid molecules. These molecules differ from the previously described MAGE nucleic acid molecules in that members of the MAGE-Xp family do not hybridize to the previously identified MAGE sequences. Further, the members of the MAGE-B family are found on the Xp arm of the X chromosome rather than on the Xq chromosome, as was the case with the previously identified MAGE genes.

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ISOLATED NUCLEIC ACID MOLECULES WHICH ARE MEMBERS OF THE MAGE-B FAMILY AND USES THEREOF

RELATED APPLICATIONS

This application is a continuation-in-part of Serial No. 08/658,578, filed June 5, 1996, which is a continuation-in-part of Serial No. 08/403,388, filed March 14, 1995, both of which are incorporated by reference.

FIELD OF THE INVENTION

This invention relates to a nucleic acid molecule which codes for a tumor rejection antigen precursor. More particularly, the invention concerns genes, whose tumor rejection antigen precursor is processed, inter alia, into at least one tumor rejection antigen. The tumor rejection antigen precursors in question do not appear to be closely related to other known tumor rejection antigen precursor coding sequences, and were isolated from the Xp region of human X chromosomes, in contrast to the genes to which they are most closely related, which were found on the Xq region. These newly isolated genes are members of the MAGE-B family, while those in the Xq region are now considered to be members of the MAGE-A family.

BACKGROUND AND PRIOR ART

The process by which the mammalian immune system recognizes and reacts to foreign or alien materials is a complex one. An important facet of the system is the T lymphocyte, or "T cell" response. This response requires that T cells recognize and interact with complexes of cell surface molecules, referred to as human leukocyte antigens ("HLA"), or major histocompatibility complexes ("MHCs"), and peptides. The peptides are derived from larger molecules which are processed by the cells which also present the HLA/MHC molecule. See in this regard Male et al., Advanced Immunology (J.P. Lipincott Company, 1987), especially chapters 6-10. The interaction of T cells and HLA/peptide complexes is restricted, requiring a T cell specific for a particular combination of an HLA molecule and a peptide. If

a specific T cell is not present, there is no T cell response even if its partner complex is present. Similarly, there is no response if the specific complex is absent, but the T cell is present. This mechanism is involved in the immune system's response to foreign materials, in autoimmune pathologies, and in responses to cellular abnormalities. Much work has focused on the mechanisms by which proteins are processed into the HLA binding peptides. See, in this regard, Barinaga, Science 257: 880 (1992); Fremont et al., Science 257: 919 (1992); Matsumura et al., Science 257: 927 (1992); Latron et al., Science 257: 964 (1992).

The mechanism by which T cells recognize cellular abnormalities has also been implicated in cancer. For example, in PCT application PCT/US92/04354, filed May 22, 1992, published on November 26, 1992, and incorporated by reference, a family of genes is disclosed, which are processed into peptides which, in turn, are expressed on cell surfaces, which can lead to lysis of the tumor cells by specific CTLs cytolytic T lymphocytes, or "CTLs" hereafter. The genes are said to code for "tumor rejection antigen precursors" or "TRAP" molecules, and the peptides derived therefrom are referred to as "tumor rejection antigens" or "TRAs". See Traversari et al., Immunogenetics 35: 145 (1992); van der Bruggen et al., Science 254: 1643 (1991), for further information on this family of genes. Also, see U.S. patent application Serial Number 807,043, filed December 12, 1991, now U.S. Patent No. 5,342,774, incorporated by reference in its entirety. The "MAGE" family of tumor rejection antigen precursors is disclosed in this patent.

In U.S. patent application Serial Number 938,334, now U.S. Patent No. 5,405,940, April 15, 1995, the disclosure of which is incorporated by reference, it is explained that the MAGE-1 gene codes for a tumor rejection antigen precursor which is processed to nonapeptides which are presented by the HLA-A1 molecule. The nonapeptides which bind to HLA-A1 follow a "rule" for binding in that a motif is satisfied.

In this regard, see e.g. PCT/US93/07421; Falk et al., Nature 351: 290-296 (1991); Engelhard, Ann Rev. Immunol. 12: 181-207 (1994); Ruppert et al., Cell 74: 929-937 (1993); Rötzschke et al., Nature 348: 252-254 (1990); Bjorkman et al., Nature 329: 512-518 (1987); Traversari et al., J. Exp. Med. 176: 1453-1457 (1992). The references teach that given the known specificity of particular peptides for particular HLA molecules, one should expect a particular peptide to bind to one HLA molecule, but not to others. This is important, because different individuals possess different HLA phenotypes. As a result, while identification of a particular peptide as being a partner for a specific HLA molecule has diagnostic and therapeutic ramifications, these are only relevant for individuals with that particular HLA phenotype. There is a need for further work in the area, because cellular abnormalities are not restricted to one particular HLA phenotype, and targeted therapy requires some knowledge of the phenotype of the abnormal cells at issue.

In U.S. Patent Application Serial Number 008,446, filed January 22, 1993 and incorporated by reference, the fact that the MAGE-1 expression product is processed to a second TRA is disclosed. This second TRA is presented by HLA-Cw*1601 molecules. The disclosure shows that a given TRAP can yield a plurality of TRAs, each of which will satisfy a motif rule for binding to an MHC molecule.

In U.S. Patent Application Serial Number 994,928, filed December 22, 1992, and incorporated by reference herein teaches that tyrosinase, a molecule which is produced by some normal cells (e.g., melanocytes), is processed in tumor cells to yield peptides presented by HLA-A2 molecules.

In U.S. patent application Serial No. 08/032,978, filed March 18, 1993, and incorporated by reference in its entirety, a second TRA, not derived from tyrosinase is taught to be presented by HLA-A2 molecules. The TRA is derived from a TRAP, but is coded for by a non-MAGE gene. This disclosure shows that a particular HLA molecule may present TRAs derived from different sources.

In U.S. patent application Serial No.08/079,110, filed June 17, 1993 and incorporated by reference herein, an unrelated tumor rejection antigen precursor, the so-called "BAGE" precursor is described. The BAGE precursor is not related to the MAGE family.

In U.S. patent applications Serial No. 08/096,039 and Serial No. 08/250,162, both of which are incorporated by reference, non-related TRAP precursor GAGE is also disclosed.

The work which is presented by the papers, patent, and patent applications cited supra deal, in large part, with the MAGE family of genes, and the unrelated BAGE, GAGE and DAGE genes, showing that there are different, additional tumor rejection antigen precursors expressed by cells.

It has now been found that there is yet another family of tumor rejection antigen precursor genes. These nucleic acid molecules show homology to the MAGE family of genes, but this homology is insufficient to identify the members of the MAGE-B family by hybridization with the members of the MAGE-A family, as set forth in, e.g., PCT Application PCT/US92/04354 and U.S. Patent No. 5,342,774, under the conditions of stringency set forth therein. Further, the isolated nucleic acid molecules of the invention were all found on the Xp arm of the X chromosome, as contrasted to the previously identified members of the MAGE-A family, all of which were found on the Xq arm. Thus, the invention relates to isolated nucleic acid molecules which encode for MAGE-B tumor rejection antigen precursors and the uses thereof.

The invention is explained in further detail in the disclosure which follows.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1

The cosmids D5 and 4965 have been described by Muscatelli, et al., Nature 372: 672-676 (1994), as well as in Muscatelli, et al., Proc. Natl. Acad. Sci. USA 92: 4987-4991 (1995) the disclosures of which are incorporated by

reference. These cosmids contain portions of the Xp arm of the X-chromosome. The cosmids were digested, using restriction endonucleases EcoRI, BamHI, Hind III, and PstI. Once digested, the DNA was transferred, to a nylon membrane, following agarose electrophoretic migration in an agarose gel.

Following this, a probe, based upon SEQ ID NO: 1, i.e., the sequence for Xp1, was used in hybridization experiments. The probe was approximately 0.45 kilobases in length, and contains 41 base pairs of the first exon (73 base pairs total), the complete second exon, and 299 base pairs of the third (1603 base pairs total). The sequence for what is referred to herein as "MAGE-B1" and is referred to elsewhere as "Xp" may be found in Muscatelli, et al., Proc. Natl. Acad. Sci. USA supra. Further the sequence is found in the EMBL sequence data bank reference to accession number emb X82539, available no later than February 7, 1995.

In order to prepare the 0.4 kb probe, the following primers, i.e., SEQ ID NO: 11 and SEQ ID NO: 12 were used, in PCR, on B1 cDNA:

5'-GTGGTGTCCAGCAGTGTCTC-3'

5'-GTCAGATTCGGTACATGACACAG-3

Specifically, the DNA was denatured with NaOH and neutralized in the gel before transfer to a nylon membrane using 20xSSC (SSC=0.15M NaCl, 0.015M sodium citrate, pH 7). Following transfer, the membranes were rinsed for 5 minutes in 6xSSC at room temperature, baked for one hour at 80°C, and pretreated for 4 hours in 6xSSC, 10xDenhardt's solution (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA), at 65°C.

The membrane was then hybridized in 3.5xSSC, 1xDenhardt's Solution, 25 mm sodium phosphate buffer (pH 7.0), 0.5% SDS, 2mM EDTA and $3x10^6$ cpm/ml α ^{32}P-CTP radiolabelled probe. Hybridization was performed for 18 hours at 65°C. The membrane was then washed at 65°C, four times, for one hour each time in 2xSSC, 0.5% SDS, 1xDenhardt's solution; once for 30 minutes at 0.2xSSC, 0.1%

SDS; and once for 30 minutes in 0.1xSSC, 0.1% SDS. The membranes were autoradiographed using Kodak X-ARS film, and Kodak X-Omatic fine intensifying screens.

Following the hybridization, several signals of differing intensity were observed. Of these, three EcoRI fragments from cosmid 4965, which were 1.5, 2.2, and 2.5 kilobases in length were isolated, and cloned into vector pTZ19R for sequencing. Partial sequencing showed that each fragment contained a sequence homologous to the third exon of B1. Homology of the three sequences, relative to B1, was 75%, 60%, and 80%, for genes referred to hereafter as MAGE-B2, MAGE-B3, and MAGE-B4. These are presented in SEQ ID NOS: 2, 3 and 4, respectively.

The foregoing disclosure, places many tools of extreme value in the hands of the skilled artisan. To begin, the examples identify isolated nucleic acid molecules which code for MAGE-B tumor rejection antigen precursors as well as the nucleic acid molecules complementary thereto. It is known that DNA exists in double stranded form, and that each of the two strands is complementary to the other. Nucleic acid hybridization technology has developed to the point where, given a strand of DNA, the skilled artisan can isolate its complement, or synthesize it. The invention includes, inter alia, the phenomenon of double strandedness to permit the artisan to identify the X chromosome, especially the Xp element, as well as defects in the chromosome.

Such assays can be carried out by one of ordinary skill in the art, using standard methodologies. For example, using the well known polymerase chain reaction (PCR), one uses the following primers:

For identifying B2:

5'-TAAAAAAGGTGCCAAGAGCCAC-3' (SEQ ID NO: 5);

5'-TGAGGCCCTCAGAGGCTTTC-3' (SEQ ID NO: 6).

For identifying B3:

5'-AGTCTGCTGGTAGGTCACGTA-3' (SEQ ID NO: 7);

5'-TCAGGAACTGCACCAACATATTT-3' (SEQ ID NO: 8).

For identifying B4:

5'-AGGGATACTGCCTCCAGCTC-3' (SEQ ID NO: 9);

5'-CAGGAACTGCACTAACATCTTC-3' (SEQ ID NO: 10).

Example 2, which follows, shows one way this can be done.

EXAMPLE 2

The primers of SEQ ID NO: 5 and SEQ ID NO: 6 were used, for example, to determine whether or not MAGE-B2 was expressed in tumors.

Total cellular RNA was extracted from tumor cell samples, using the well known guanidine-isothiocyanate/cesium chloride methodology, (see, e.g., Davis et al., Basic Methods in Molecular Elsevier, NY (1986), pp. 130-135, which is not repeated here. Next, cDNA was synthesized, using 2 ug total RNA from the samples. Synthesis was carried out by extension with oligo dT(15), in a 20 μl reaction volume, in accordance with DeSmet et al, Immunogenetics 39: 121-129 (1994), incorporated by reference. After incubation for one hour at 42°C, the cDNA reaction mixture was diluted with water to 100 μl. PCR was then carried out using SEQ ID NOS: 5 and 6. Each PCR reaction was carried out, using 5 μl of cDNA (which corresponds to 100 ng of RNA), supplemented with 5 μl of 10xPCR buffer, and 1 μl of each variety of dNTP (10 mM), 0.5 μl each of 80μ M solutions of primers, 1.25 units of AmpliTaq DNA polymerase and water, to a total volume of 50 μl. This mixture was then heated to 94°C for five minutes, followed by amplification in a thermal cycler for 30 cycles (one minute at 94°C, two minutes at 63°C, two minutes at 72°C). Cycling was then concluded with a final extension step (15 minutes, 72°C). A 10 μl sample of each reaction was run on 1% agarose gel, and visualized using ethidium bromide fluorescence.

RNA integrity was verified, and samples containing strongly degraded RNA excluded, by carrying out a 20 cycle PCR assay, using primers specific for ß-actin, in accordance with Weynants et al, Int. J. Cancer 56: 826-829 (1994) incorporated by reference.

The results for tumors follow. The first column is the number of tumor samples tested, the second is the number which were positive for MAGE-B2:

Testicular seminoma	6	5
Non-small cell lung carcinoma	20	6
Melanoma	26	5
Breast	10	2
Sarcoma	10	1
Leukemia	10	1

With the exception of the positive leukemia, any tumor sample which was positive for MAGE-B2 was also positive for at least one MAGE-Xq.

Expression of MAGE-B2 was found in fetal and adult testis, but was not found in any normal kidney, liver, adrenal gland, skin, breast, brain, heart, ovary, prostate, cerebellum, peripheral blood lymphocyte, colon, stomach, lung, bladder, bone marrow or endometrium cells.

EXAMPLE 3

Additional experiments were carried out on cosmids D5 and 4965, which are discussed in example 1, supra. Specifically cDNA as disclosed by Muscatelli, et al, Proc. Natl. Acad. Sci USA 92:4987-4991 (1995), was subjected to PCR amplification. In these amplifications, the primers:

5'- GTGGTGTCCA GCAGTGTCT C -3' (SEQ ID NO:11)

and

SEQ ID NO:12

were used, to generate a 0.45kb probe. A second probe was then prepared using:

5' - AAT GTG TTG GGA GCC TAT GAT -3' (SEQ ID NO:13)

and

5' - ATT ATG TTG TGT GAG GTT CTT TCA -3' (SEQ ID NO:14)

to generate a 726 base pair probe.

The first probe contained 41 bp of exon 1, 105 bp of exon 2, and 300 bp of exon 3 of MAGE-B1, while the second probe consisted of the 726 bp at the 3'-end of exon 4.

Southern blotting was then carried out on both cosmids, using standard methods as can be found in, e.g., Lurquin, et

al, Cell 58:293-303 (1989). Any fragments of the cosmids which hybridized with the probes were cloned into commercially available vectors (ethyl pTZ18R or pTZ19R), and then sequenced.

The results of this work identified three sequences which showed significant identity to the last exon of MAGE-B1, as reported by Muscalelli, et al, supra. One sequence was identical to MAGE-B2, as described in Lurquin, et al, U.S. Patent No. 5,587,289, as MAGE-Xp2, and by Dabovic, et al, Mamm. Genome 6:571-580 (1995), as "DAM 6". This meant that there were two other homologous genes present in the cosmids.

EXAMPLE 4

In order to determine the precise positions, and complete sequences of the positives described supra, the portion of the Xp arm of the X chromosome, found in cosmids D5 and 4965, that includes the sequences of these hybridizing fragments was sequenced by "chromosome walking" (as described in Molecular Biology of the Cell, Alberts et al., Second Edition p.262-265).

A total of 40,352 kb was sequenced and this complete sequence is set out in SEQ ID NO:15. No further sequencing was carried out after this 40.352 kb sequence has been obtained because the start site and 5' UTR of MAGE-B2 was at the 5' end of this 40.352 kb sequence and the stop condon and poly-A signal of MAGE-B1 was located at the 3' end of this 40.352 kb sequence. At this point it was clear that all of the Xp hybridizing fragments from the Southern analysis (described in Example 3) were located within this 40,352 kb sequence obviating the need for any further sequencing.

When the sequence information obtained in example 3 was compared to the full, 40,352 bases of SEQ ID NO:16, the following was discovered:

GENE	POSITION IN SEQ ID NO:15
B2	3266 - 7979
B3	23546 - 25194
B4	29748 - 31474

B1 31403 - 39691

Within these sequences, further analyses showed that B2 contains two exons, at nucleotides 3266-3364, and 6278-7979, respectfully. The entire coding region is found at nucleotides 6283-7224, with a poly A signal being found at nucleotides 7961-7966.

As to the B3 gene, a single coding exon, at nucleotides 23546-25194 was found. The coding region consisted of nucleotides 23607-24647, with a poly-A signal at nucleotides 25152-25157.

The gene for B4 is thought to extend through to poly-A signal at 31822-31827, with the coding sequence being found at nucleotides 29808-30848.

The MAGE-B1 gene is the most complex of the four. The first exon, at nucleotides 31403-31474, is within the MAGE-B4 coding exon. Exons 2, 3 and 4 are found at nucleotides 33958-39691, i.e., at 33958-34062, 35057-35139, and 38088-39691, respectively. The coding sequence is found completely within the fourth exon, i.e., at nucleotides 38148-39191. The poly-A signal is at 39674-39679.

EXAMPLE 5

Comparison of the nucleotides in these sequences and other known tumor rejection antigen precursors, is set forth in Table 1, which follows. It can be seen that MAGE-B1, B2 and B4 form a closely related set, with about 80% identity while MAGE-B3 is about 70% identical with the others.

Further comparison reveals protein encoding regions corresponding to 347, 313, 346, and 346 amino acids for the MAGE-B proteins. These show anywhere from 49-68% identity.

Table 1. Sequence comparison of the human and mouse MAGE coding regions and proteins

% Nucleotide Identity

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	B1	B2	B3	B4	sB1	sB2	sB3
MAGE-A1	100	80	81	84	81	81	84	77	76	68	75	81	62	69	61	80	58	97	96
MAGE-A2	80	100	92	82	82	92	91	78	74	65	73	83	59	60	59	81	86	88	96
MAGE-A3	81	92	100	82	80	88	80	78	78	68	75	82	61	61	60	82	84	84	94
MAGE-A4	84	82	82	100	87	83	84	79	79	69	77	83	63	61	61	82	87	87	97
MAGE-A5	81	82	86	87	100	86	74	77	73	80	74	84	50	45	62	47	51	51	86
MAGE-A6	81	92	88	83	86	100	80	776	76	68	76	93	61	61	60	81	64	64	64
MAGE-A7	84	91	80	84	74	80	100	83	87	70	79	81	54	56	54	82	46	48	43
MAGE-A8	77	78	78	79	77	78	83	100	78	68	76	76	60	60	58	61	47	47	46
MAGE-A9	76	74	78	78	73	78	87	79	100	69	78	77	62	58	61	83	67	57	56
MAGE-A10	68	65	68	69	80	68	79	88	89	100	72	88	60	60	68	83	68	58	58
MAGE-A11	76	73	75	77	74	76	78	78	76	72	100	75	62	62	62	85	60	58	59
MAGE-A12	81	83	82	83	84	82	81	78	77	88	76	100	62	60	60	82	56	60	67
MAGE-B1	62	59	61	63	60	61	54	60	62	60	62	62	100	80	66	81	83	83	83
MAGE-B2	59	60	61	61	46	61	56	59	58	60	62	60	80	100	66	76	84	84	84
MAGE-B3	61	59	60	61	62	60	54	56	61	62	62	60	66	66	100	71	80	80	81
MAGE-B4	80	81	82	82	87	81	82	81	83	83	83	82	81	78	71	100	81	81	81
Smage-B1	58	65	64	67	61	64	68	67	67	66	66	66	63	64	60	81	100	99	98
Smage-B2	97	96	84	87	81	84	88	87	87	68	68	66	63	64	60	81	99	100	98
Smage-B3	96	88	84	87	86	84	85	88	88	68	68	67	63	64	61	81	98	98	100

% Amino Acid Identity

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	B1	B2	B3	B4	sB1	sB2	sB3
MAGE-A1	100	87	87	76	68	88	23	84	80	52	59	87	38	38	43	43	36	36	37
MAGE-A2	87	100	84	87	88	84	18	82	89	46	58	86	39	38	38	40	33	33	33
MAGE-A3	87	84	100	87	72	86	17	82	90	47	60	86	37	37	34	38	33	33	34
MAGE-A4	76	87	87	100	78	87	23	86	84	61	82	87	42	39	41	41	36	38	38
MAGE-A5	68	88	72	78	100	72	13	82	82	39	60	69	28	30	20	24	10	10	20
MAGE-A6	88	84	86	87	72	100	18	82	88	49	80	84	37	37	35	40	34	34	34
MAGE-A7	23	18	17	23	13	18	100	25	27	20	21	17	16	16	20	14	13	13	14
MAGE-A8	84	82	82	86	81	82	25	100	86	84	80	84	38	35	38	38	20	20	20
MAGE-A9	80	89	88	84	82	88	27	86	100	50	89	89	39	38	38	43	34	34	34
MAGE-A10	68	46	47	61	30	48	20	84	80	100	50	48	41	38	41	47	36	38	36
MAGE-A11	89	88	88	82	89	88	21	88	89	88	100	88	42	38	40	44	38	38	38
MAGE-A12	87	88	88	87	88	84	17	84	88	45	88	100	39	37	36	40	33	33	34
MAGE-B1	38	39	37	42	28	37	18	38	38	41	48	38	100	82	49	88	47	47	47
MAGE-B2	38	38	37	39	30	37	16	33	38	38	38	37	82	100	49	83	48	48	47
MAGE-B3	42	38	34	41	30	38	20	38	38	41	40	38	49	49	100	66	42	42	43
MAGE-B4	43	40	38	41	24	40	14	38	43	47	44	40	88	83	95	100	82	82	81
Smage-B1	38	38	33	38	19	34	13	28	34	38	38	33	47	48	48	82	100	100	97
Smage-B2	36	88	33	38	19	34	13	28	34	36	38	33	47	48	42	82	100	100	97
Smage-B3	37	38	34	38	20	34	14	28	34	36	39	34	47	47	43	81	97	97	100

EXAMPLE 6

In work reported by Muscatelli, et al, Proc. Natl. Acad, Sci. USA 92:4987-4991 (1995), MAGE-B1 from a cDNA library from testis was found to comprise two types, i.e.,

one included all four exons, and the other, exons 1, 2 and 4.

Experiments were carried out to verify this, using SEQ ID NOS: 11 and 12, set forth, supra, on a testis cDNA library, using RT-PCR. To carry this out, total cellular RNA was extracted, using the well known guanidine - isothiocyanate/cesium chloride method of, e.g., Davis, et al, Basic Methods In Molecular Biology, Elsevier Science Publishing Co., Inc., New York (1986). Samples (2μg), of total RNA were used for cDNA synthesis, via extension of oligo dt(15), in 20μl reaction volumes. See DeSmet, et al, Immunogenetics 39:121-129 (1996). The cDNA was incubated at 42°C, for 1 hour, and then diluted to 100μl with water. The primers set forth, supra, were then combined with 5μl of cDNA, together with 5μl of 10xDNA polymerase buffer, 1μl of each of 10mM dNTP, ad 1 unit of .DNA polymerase. Water was added to a total volume of 50μl. The mixture was heated to 94°C for 5 minutes, followed by amplification for 30 cycles (a cycle: 1 minute 94°C, 2 minutes at 63°C, and an extension of 2 minutes at 72°C). The cycling was concluded with a final extension step of 15 minutes at 72°C. Following this, a 10μl sample of the reaction was run on a 1.5% agarose gel, and visualized by ethidium bromide fluorescence. RNA integrity was verified, and samples with strongly degraded RNA, were excluded by carrying out a PCR assay of 20 cycles, using B-actin specific primers.

The results verified the previous findings, that there were two types of transcript which were present. The transcript containing 4 exons was far less abundant than the other.

The pattern of amplification products using SEQ ID NO:17 and 12 was also determined using RT-PCR on a testis cDNA library. In addition to a species comprising all 4 exons, a major species containing exons 3 and 4 was obtained.

Eighty-four tumor samples and tumor cell lines of various histological types were found to be negative for

MAGE-B1 expression when tested with primers whose sequences were located in the first and fourth exons (SEQ ID NOS:11 and 12). However, using primers whose sequences were located in the third and fourth exons (SEQ ID NOS:17 and 12), MAGE-B1 expression was detected in samples from NSCLC and mammary carcinoma and tumors of other histological type patients.

EXAMPLE 7

The pattern of distribution of expression of the MAGE-B genes was studied, via RT-PCR.

The protocol set forth in example 5, supra, was followed with some changes, as indicated herein.

Various combinations of primers were used, based upon the MAGE-B sequences. In addition to SEQ ID NO: 11 and 12, presented supra, the following primers were used for MAGE-B1:

5′-GAT CAT CCA GGA GTA CAA CTC GA -3′ (SEQ ID NO:16)

5′-CCC GAG CGA GCT TAA GGA GT -3′ (SEQ ID NO:17)

SEQ ID NOS: 11, 16 and 17 are sense primers corresponding to 1, 2 and 3, respectively, of MAGE-B1. One of these was used in combination with SEQ ID NO:12, in assays for expression of MAGE-B1.

For MAGE-B2, one of

5′ -AGC GAG TGT AGG GGG TGC G -3′ (SEQ ID NO:18) or SEQ ID NO:15, supra, together with SEQ ID NO:6, supra, were used. SEQ ID NOS:5 and 18 are sense primers for exons 1 and 2 of MAGE-B2, while SEQ ID NO:6 is an antisense primer for exon 2.

As indicated, RT-PCR was carried out, essentially as in Example 5, with the following exceptions. Forty cycles were carried out for MAGE-B1, while MAGE-B2 was assayed using thirty cycles. The cycle parameters given in example 5, supra, was modified as follows. When SEQ ID NOS:17 and 12, and SEQ ID NO:18 and 6, were used, a cycle was 1 minute at 94°C, and 2 minutes at 68°C, followed by the two minute extension. When SEQ ID NOS: 16 and 12 were used, the two minutes was carried out at 65°C.

The results are set forth in Table 2, which follows:

	Mage-B1 LUR171-1338 40 cycles	Mage-B2 LUR84-LUR85 and/or LUR162-LUR85 30 cycles
Surgical tumor samples		
Colorectal carcinoma	0/12	0/12
Gastric carcinoma	0/2	0/2
Leukemia	0/48	1/50
Myeloma	0/1	0/1
Melanoma	8/36	8/37
Skin carcinoma	1/4	0/4
Naevus (benign lesion)	0/8	0/6
Brain tumor	0/8	0/8
Neuroblastoma	0/2	0/2
Head and neck squamous cell carcinoma	0/12	2/12
Pleural mesothelioma	0/3	0/3
Small cell lung carcinoma	0/1	0/1
Non-small lung carcinoma	4/29	13/29
Sarcoma	1/11	2/11
Mammary sarcinoma	2/12	3/12
Prostate adenocarcinoma	0/6	0/6
Testicular tumor	8/9	8/9
Renal cell carcinoma	0/11	0/11
Bladder carcinoma	0/12	0/12
Cell lines		
colorectal carcinoma	0/5	0/5
Leukemia	0/3	0/3
EBV transformed B lymphocytes	0/1	0/1
Melanoma	2/9	3/9
Small cell lung carcinoma	0/2	1/2
Non small cell lung carcinoma	0/6	3/6
Sarcoma	0/2	0/2
Normal tissues		
Colon	0/1	0/1
Stomach	0/1	0/1
Liver	0/1	0/1
Bone marrow	0/1	0/1
Peripheral blood lymphocytes	0/1	0/1
Thymocytes	0/1	0/1
Skin	0/1	0/1
Brain	0/2	0/2
Cerebellum	0/1	0/1
Heart	0/1	0/1

Lung	0/1	0/1
Breast	0/2	0/2
Ovary	0/1	0/1
Uterus	0/2	0/2
Prostate	0/1	0/1
Testis	2/2	2/2
Adrenal gland	0/1	0/1
Kidney	0/1	0/1
Bladder	0/1	0/1
Fetal tissues: liver	0/1	0/1
brain	0/1	0/1
testis	1/1	1/1
placenta	0/1	1/1

Note that, in this table and the table which follows "LUR 171" is SEQ ID NO:17, "1338" is SEQ ID NO:12, "1339" is SEQ ID NO:11, "LUR 162" is SEQ ID NO:18, "LUR 84" is SEQ ID NO:5 and "LUR 85" is SEQ ID NO:6.

EXAMPLE 8

It is known that certain MAGE genes are inducible with 5-aza-2'- deoxycytidine, in both melanoma cells, and in different cell types which do not normally express the genes. See Weber, et al, Cancer Res 54:1766-1771 (1994); DeSmet, et al, Proc. Natl. Acad Sci. USA 93:7149-7153 (1996); DePlaen, et al, Genomics 40: (1997). Additional agents may also be used to induce MAGE genes.

In order to determine if the MAGE-1 genes parallel other genes in terms of inducibility, different types of cells were incubated for 72 hours in culture medium containing 1μm 5-aza-2'-deoxycytidine ("DAC" hereafter), in accordance with DeSmet, et al, supra. The table which follows sets forth the result.

	MAGE-B1				MAGE B-2	
	LUR171-1338 (exon3-exon4)		1339-1338 (exon1-exon4)		LUR162-LUR85 (exon1-exon2)	
	-	+DAC	-	+DAC	-	+DAC
Cell lines:						
MZ2-MEL	-	+	-	-	-	+
SK23-MEL	-	-	-	-	-	-

M1665/2-MEL	-	+	-	-	-	+
LE92.11-RCC	-	-	-	-	-	+
JAR	-	+	-	-	-	+
LB23-SAR	-	+	-	-	-	+
B-EBV	-	+	-	-	-	+
Normal tissues:						
PBL-PHA	-	+	-	-	-	+
Fibroblasts	-	-	-	-	-	+
Dentritic cells	-	+	-	-	-	+

"Nucleic acid molecule" as used herein refers to all species of DNA and RNA which possess the properties discussed supra. Genomic ("gDNA") and complementary DNA, or "cDNA" both code for particular proteins, and as the examples directed to isolation of MAGE coding sequences show, this disclosure teaches the artisan how to secure both of these.

The four MAGE-B genes are spread over 40,352kb in the 160kb X-linked critical region defined for the DSS (Dosage Sensitive Sex reversal) locus involved in sex determination (Bardoni et al. Nature Genetics 7:497-501 (1994)). This region is duplicated in patients with a male-to-female sex reversal phenotype. Genes in this region may be involved in X-linked disorders such as adrenal hypoplasis congenita and hypogonadism.

All isolated nucleic acid molecules which encode MAGE-B proteins, with the exception of MAGE-B1, are encompassed by this invention. This includes those nucleic acid molecules which hybridize to any of MAGE-B2, MAGE-B3, or MAGE-B4 under stringent conditions. As used herein, this refers to conditions such as hybridization with $5x10^6$ cpm/ml for 18 hours at 65°C, followed by 4, 20 minute washes at 65°C, with each wash using 2xSSC, 0.5% SDS and 1xDenhardt's solution, followed by two washes at 0.2xSSC, 1% SDS (20 minutes, each wash), and, finally, two washes at 68°C, 1% SDS, a varying concentration of SSC, each of these washes being for 20 minutes. The final concentration of SSC should be no greater than 0.5xSSC, more preferably it is 0.2xSSC, and most preferably it is 0.1xSSC.

Similarly, RNA molecules, such as mRNA can be secured. Again, with reference to the skilled artisan, once one has a coding sequence in hand, mRNA can be isolated or synthesized.

Complementary sequences which do not code for TRAPs, such as "antisense DNA" or mRNA are useful, e.g., in probing for the coding sequence as well as in methodologies for blocking its expression.

It will also be clear that one may manufacture biologically pure cultures of prokaryotic and eukaryotic cell lines which have been transformed or transfected with nucleic acid sequences which code for or express the MAGE-B molecules. Such cultures can be used as a source for tumor rejection antigens, e.g., or as therapeutics. This aspect of the invention is discussed infra.

Cells transfected with MAGE-B coding sequences may also be transfected with other coding sequences. Examples of other coding sequences include cytokine genes, such as interleukins (e.g., IL-2 or IL-4), or major histocompatibility complex (MHC) or human leukocyte antigen (HLA) molecules. Cytokine gene transfection is of value because expression of these is expected to enhance the therapeutic efficacy of the biologically pure culture of the cells in vivo. The art is well aware of therapies where interleukin transfectants have been administered to subjects for treating cancerous conditions. In a particularly preferred embodiment, cells are transfected with sequence coding for each of (i) MAGE-Xp molecule, (ii) an HLA/MHC molecule, and (iii) a cytokine.

Transfection with an MHC/HLA coding sequence is desirable because certain of the TRAs derived from MAGE-B may be preferentially or especially presented only by particular MHC/HLA molecules. Thus, where a recipient cell already expresses the MHC/HLA molecule associated with presentation of a TRA, additional transfection may not be necessary although further transformation could be used to cause overexpression of the antigen. On the other hand, it

may be desirable to transfect with a second sequence when the recipient cell does not normally express the relevant MHC/HLA molecule. It is to be understood, of course, that transfection with one additional sequence does not preclude further transfection with other sequences.

The term "biologically pure" as used in connection with the cell line described herein simply means that these are essentially free of other cells. Strictly speaking, a "cell line" by definition is "biologically pure", but the recitation will establish this fully.

Transfection of cells requires that an appropriate vector be used. Thus, the invention encompasses expression vectors where a coding sequence for the MAGE-Xp TRAP of interest is operably linked to a promoter. The promoter may be a strong promoter, such as those well known to the art, or a differential promoter, i.e., one which is operative only in specific cell types. The expression vectors may also contain all or a part of a viral or bacterial genome, such as vaccinia virus or BCG. Such vectors are especially useful in preparing vaccines.

The expression vectors may incorporate several coding sequences, as long as the MAGE-B sequence is contained therein. The cytokine and/or HLA genes discussed supra may be included in a single vector with the TRAP sequence. Where this is not desired, then an expression system may be provided, where two or more separate vectors are used where each coding sequence is operably linked to a promoter. Again, the promoter may be a strong or differential promoter. Co-transfection is a well known technique, and the artisan in this field is expected to have this technology available for utilization. The vectors may be constructed so that they code for the TRA molecule directly, rather than the MAGE-Xp TRAP. This eliminates the need for post-translational processing.

As the foregoing discussion makes clear, the sequences code for "tumor rejection antigen precursors" ("TRAPs") which, in turn, are processed into tumor rejection antigens

("TRAs"). Perhaps their most noteworthy aspect is as vaccines for treating various cancerous conditions. The evidence points to presentation of TRAs on tumor cells, followed by the development of an immune response and deletion of the cells. The evidence in the art shows that when various TRAs are administered to cells, a CTL response is mounted and presenting cells are deleted. This is behavior characteristic of vaccines, and hence TRAPs, which are processed into TRAs, and the TRAs themselves may be used, either alone or in pharmaceutically appropriate compositions, as vaccines. Similarly, presenting cells may be used in the same manner, either alone or as combined with ingredients or yield pharmaceutical compositions. Additional materials which may be used as vaccines include isolated cells which present the TRA molecule on their surface, as well as TRAP fragments, mutated viruses, especially etiolated forms, and transformed bacteria. "Fragments" as used herein refers to peptides which are smaller than the TRA, but which possess the properties required of a vaccine, as discussed supra. Another vaccine comprises or consists of complexes of TRA and HLA molecule. Vaccines of the type described herein may be used preventively, i.e., via administration to a subject in an amount sufficient to prevent onset of a cancerous condition.

The generation of an immune response, be it T-cell or B-cell related, is characteristic of the effect of the presented tumor rejection antigen. With respect to the B-cell response, this involves, inter alia, the generation of antibodies to the TRA, i.e., which specifically bind thereto. In addition, the TRAP molecules are of sufficient size to render them immunogenic, and antibodies which specifically bind thereto are a part of this invention. These antibodies may be polyclonal or monoclonal, the latter being prepared by any of the well recognized methodologies for their preparation which need not be repeated here. For example, mAbs may be prepared using an animal model, e.g., a Balb/C mouse or in a test tube, using, e.g., EBV

transformants. In addition, antiserum may be isolated from a subject afflicted with a cancerous condition where certain cells present a TRA. Such antibodies may also be generated to epitope defined by the inter-action of TRA and HLA/MHC molecules.

Review of the foregoing disclosure will show that there are a number of facets to the system which may be referred to as "tumor rejection antigen presentation and recognition". Recognition of these phenomena has diagnostic consequences. For example, the existence of specific CTL clones, or antibodies to the TRA makes it possible to diagnose or monitor cancerous conditions (explained infra), by monitoring the CTLs in a sample from a subject, binding of antibodies to TRAs, or the activity of anti-TRA CTLs in connection with subject samples. Similarly, the expression of nucleic acid molecules for TRAPs can be monitored via amplification (e.g., "polymerase chain reaction"), anti-sense hybridization, probe technologies, and so forth. Various subject samples, including body fluids (blood, serum, and other exudates, e.g.), tissues and tumors may be so assayed.

A particular manner of diagnosis is to use an adaptation of the standard "tuberculin test" currently used for diagnosis of tuberculosis. This standard skin test administers a stable form of "purified protein derivative" or "PPD" as a diagnostic aid. In a parallel fashion, TRAs in accordance with this invention may be used in such a skin test as a diagnostic aid or monitoring method.

The term "cancerous condition" is used herein to embrace all physiological events that commence with the initiation of the cancer and result in final clinical manifestation. Tumors do not spring up "ab initio" as visible tumors; rather there are various events associated with the transformation of a normal cell to malignancy, followed by development of a growth of biomass, such as a tumor, metastasis, etc. In addition, remission may be conceived of as part of "a cancerous condition" as tumors

seldom spontaneously disappear. The diagnostic aspects of this invention include all events involved in carcinogenesis, from the first transformation to malignancy of a single cell, through tumor development and metastasis, as well as remission. All are embraced herein.

Where "subject" is used, the term embraces any species which can be afflicted with a cancerous condition. This includes humans and non-humans, such as domesticated animals, breeding stock, and so forth.

There are therapeutic aspects of this invention as well. The efficacy of administration of effective amounts of TRAPs and TRAs as vaccines have already been discussed supra. Similarly, one may develop the specific CTLs in vitro and then administer these to the subject. Antibodies may be administered, either polyclonal or monoclonal, which specifically bind to cells presenting the TRA of interest. These antibodies may be coupled to specific antitumor agents, including, but not being limited to, methotrexate radio-iodinated compounds, toxins such as ricin, other cytostatic or cytolytic drugs, and so forth. Thus, "targeted" antibody therapy is included herein, as is the application of deletion of the cancerous cells by the use of CTLs.

The terms and expression which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expression of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

MAGE-B Cluster Sequence

SEQ ID NO: 17

10	20	30	40	50	60	70	80	90	100	
1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	
AAGCTTCAGA	GTTGAGAGAC	TAAGCCTATA	ATGGAAAAGT	GCTCTTAACT	TTGGAGAAAA	CTGGAAAGAA	GCTCCACCAG	GGGCAGCACT	GGAAAGTGAC	100
TATGCTCTTG	TCCCAGTGCA	GATGAACACA	GTCCAGAAAC	TAGCTGCTTT	ATATACATTG	TATCCAGTGA	GCTTCGGAGA	AATAAGGATG	ATACTCCCTT	200
AAGAAGAAAT	GATAAGAAGC	CGCTGGGTAC	TCTGCACTGG	GCTGGAAGAG	TTAAGAGTCA	ATTTCATTAA	AAGGAGCATT	CAAATTAGGC	TATCATGTAC	300
ATAATTTGGC	AAGCTCTAGG	CAAGTTCTAG	GTGTTTGGTG	CTTGTTAAAT	TAATGAAAAT	AAAGGTGATT	TAGATGGAAG	TGACACTGGG	AGGGAAGGAA	400
GTTGTTAGTC	CTTGACGCAA	ATGGGTTGTG	TTGCAGCTAA	ACTGGAGAAG	TCTACCTCAC	ATTGAATAAT	TTATCATTTA	ATGGGGAAAT	ATTACTTGAT	500
TTTTGTGCT	AATCTGCATT	TTGGCTTATT	GGGTTTTCTT	TCTTCTAGAA	TGCTGCATAT	TCTCTGACAT	CTGTTGGATT	AAGAAAAAGC	AATCACAGTG	600
GGATGGGTGG	TATCATCAAG	TTAATCATAG	GTAATAGCTG	CCATGTTGAA	ACCTCAATAT	GGGCCAAGTA	TTGTGCCAGG	TGCTTTACAT	ACATTAACCA	700
CATTTCACCA	ATTCCACAAG	GAAGGGGCTT	ATCAAAGCTA	TTTCACAGAT	GCAGAGCCTG	AGGCTCACAG	AGTTTAGTAA	TGTGCCTGAG	TTCACATAGC	800
TGGTAAGTGG	CAGGGCTGAA	ACCAGACCCC	CAGTCTGGAT	TAATCTACAT	CCCACACTGT	TCCAGTCTTC	CCATTCTGAG	GCTAACCTCA	TGTTGCTTCA	900
TTCATTTTTT	CTCTCTATTT	TTGAAAAATG	TATCTCAGGC	AATAAAAAGA	AGGACTCTGT	GCCCATACTA	CTGGGTCAAA	TATATTCCCA	GACCAATATG	1000
AATGCCAAAA	TAAATGAGAG	GCATAAATAA	AAAAAGAAA	AATAGTACCT	GGTGTCAATA	CGTTCATCTA	CACAAGCAAT	ATCAGGTAAT	CTCAAAAGAT	1100
CAGAAGCTCC	CAAAATCATC	CTGGACAGTC	TCTTCCCTGT	AGAAATAAA	TGTGTCAGAA	CAGAATTTTC	GTGAGAATCT	AAGTCTAGCA	AGATGGAAAA	1200
AATCAGCATG	TTTTTTTCTC	TGACAGCCCA	CTACCTGTCC	TGGAGCTTCT	GCCTTGTGCT	TCAGCTTCCC	TATCTCACAT	CACTCCCGGA	ACACGGCTCC	1300
TTCTCTGCAA	GGTTTGCAGT	AGAAAAACTG	GTCATGTTTG	CAGGGATGTG	ACATTTCCCA	GAAAGCCTTT	AATGAAAGAT	GATCCCCAGT	TCAGAGGACA	1400
TGGGAAACCA	ATATCTCAGA	ATAATATGAGG	TTATGCATAG	ACTGACTCTC	ATCTGCTCTC	AGACCTAGAG	AGCTGGAGAC	AGAACTGTCA	GGCTGAGCAT	1500
CCACTCATTT	AATCTTCAGA	CCTCTCATGG	ACATGGCCTA	AGAAGGTCAA	CTTCATGTCA	GTAGATGCAG	TTCAGCCTCT	GATAGATATG	AACGTGAGGA	1600
CCCTGAATGA	AGATGATGGA	AGGACCCAGC	CAGAACTGTG	GGGTCCCAAA	AAGTCCAGCT	CAGGCTGTCA	GCCCTTCAAA	GTCCAGAACA	GCCCTGGCTA	1700
AATGTACCTC	ACCACAACTT	CCAGTTCAGA	GGTCGGCAGA	GGGTGAGTAC	CTTGATCTGA	TGGGAACAGC	CTCAGGTCAG	TGGAGGATGG	AGTCGCCCAA	1800
GACTGGCCAG	GTGTCATGGT	GAGGACCCTG	AAGAGAAATA	AGAGAACTGT	TATAGGACCA	ACGAGTTTCT	GTGTCAGCTG	CAAAGTAACA	GACCCACTAT	1900
AATAAAACAG	CAAAGTTTGC	AGCAAAGAGT	TTAATGATTG	CAGGGTGCCA	AGTGAGGAGA	TGAGAAGAGA	CTCTCAAATT	CATCTCCCCA	AGGAGTTCTG	2000
GGCTGGGATT	TGCAAGGAGA	TCATGGAGGG	CGAGCGGCTG	GAGAACTGAG	GTTGTTGATT	CGTTGGGGTA	AAGGGGATGA	AATCATCAGA	ATGTGGAAAC	2100
TGCGTTTTTT	TTTTTTTAGT	CAGCTCCTGT	GGGGTCTTTT	GGACCAGCTG	ATGTCAGTAG	GGTCCTTTAG	AGCAGCTGGC	ATCAGTGGGG	TCCTTCAGAC	2200
CAGCTGAGTC	ATTAGAATCA	TCAGTATGCA	GGACCTTAAG	GAATATCTCA	AAGAAAAAAA	GTTTCATTAT	GTTAAAATTG	TTATCTATAG	AGCAATTAAG	2300
GGCAACTATA	ATCTAGTAAC	AGGATCTACA	TGATTCTGGG	ATAATAGGCA	CCAAACTACT	ATGAGGAAGC	AGGTCAGAGA	GCAGCTGACT	TCATGATTAA	2400
TGCTGAATGT	TCTGCAAGCT	TGGCGTATCT	TCATTTATCT	CCCTTCCCTC	TTCCCTGATT	AATTTTATAC	AGTTCATAGG	GGCAGTTTCA	AAACCACCCA	2500
CCCAGAACAG	AGGAGCCCAC	AAATTCCACC	AATCTGTCAC	CCTTGGGGAG	CCCAGAAGAG	AATTATGAGG	TGGAGAAATC	CCATCAATAT	GCCTCGTGTG	2600
TTTCACAGAG	GTGAAGACCT	TGGACTAGTG	GAAATGGCCC	TAGGTCAAAG	AGACAAAACC	CACGCCCTAA	CAGGAATCAA	GGTGAGGATC	CTAAGTGTTA	2700
CGAGGGGGCT	CTCCCCCTTG	CAACACCAGC	AGAGGGGCCC	AAACAGAGCC	CTCGCCATTG	TTAGCACCGA	GAGCCCCAAA	ATAAGTATCT	CTGAATCCCT	2800
TCATTCCACC	TGTATAGTCT	CGGGGAAGGA	AGGGCCTTTG	TCTGAAGAGG	GTGACCCAGT	TGTGCAGAGA	GAGAGTCTTG	GCTTTCACGG	GAATCAAGGT	2900
GAGGACTCTG	AGGGCGGATG	AGAAGACCTC	TCCCCAAAAA	AGGCACATTC	ACAGAGCCCT	GCCGCTGCTG	TCAGGCCTGT	GAGGCCAGGC	AGGGGTGGCC	3000
TGTTTGGCAC	GCTTAGATTT	CCACAGTGGG	GGCTGAGGGA	GGTGGGGGTA	TTGTTTGGAG	GCTGGCGGAT	TTGGGTCAGC	ACGCATATTC	GTCCCAGGCT	3100
GCTAGATACT	GAGGTGAGGA	CCCTAGTGGA	GACGAAGGGA	CCAGCAACGC	TAGAACAGTG	ACGTCCGGTA	GCGTCCAGCC	GTCAGCCCCT	CAGACGCCAC	3200

MAGE-B Cluster Sequence

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        10         20         30         40         50         60         70         80         90         100
1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
GGGCTGCCGG ATGTGAGTCA TCCTGACTTC CGCTTTGAAA AAAAAGACCC GAGCGGATGT GGCTCATCCT GACTTCCGCT TTGGAGGCGA GGACCCGAGC  3300
GAGTGTAGGG GGTGCGGCGT CTGGTCAGCC AGGGGTGAAT TCTCAGGACT GGTCGGCAGT CAAGGTGAGG ACCCTGAGTG TAAACTGAAG AGACCACCCC  3400
CACCCTGTAAC AAAGAGGGCC CCACTAAGTC CCGCTTCTGC ATTTGGTCCT GAGAGGCTCC GGTAAAGCCG TCCGGCAATG TTCCACCTGG AAAGTTCCAG  3500
GGCAGGGGAA GGGTGGGGGG AGGGGCAGTC GCGGGGGAAG GAGGTTTGGA CGCAGGGAAT AGGCCTCATT CTGCACGTAG GGTGGGTGTA GGCCCTAACT  3600
GAAATCAATT TGAGGCCCCT AAATGTGGAC TGATGAGAAC ATCTCCTACC CTTAACAAAG GTGCCCTCAC TAAGTCTCGT CCCTGAAGAC GGCTCTGGGA  3700
GGCCCCAGCA AAGCTGTGCC TGGAAAGTCT CAGGGAGGGG AGGGCCTTGA ACCTAGGAAG CAGCCCTGCT TCTGCTCATA TGGCATCAAC CTAAAACCTA  3800
ACTGGGGTTA AGGTGAGGAC TCTGAGTATT AATGAGCGGA CCTCTACCCA AAAGAGGAGT CATGCTGGGC AGTTCCCAGG CCGAGGTGAC ACTGGGTTTT  3900
AGATGGTCCC AGGCTGACAT GACAAATGCA GAGCAACCTG ACTTCCTCTC CCCCGACTAA AGAAGTTGAG GGCTACATCC AAGACCCACA TCTGCTGCCA  4000
GCAATAGGAA GGTCAGGGCA AAGCTAGCAA GCTGAGATGC TCTCTAGTTT CCTCTGGAAG GTGGTCTCAG GGAGGTGAGG GTCTGGTGTA TGGGGTAGGT  4100
CTAATGCGAC AGAGAGGCCC AGTCTCTAAC AGGAAGCAAG GATGTGACAC TGAGTGATGA GGAGGGGACT CCAACCAGAA AGAAGTGCCA TATAGAGCCC  4200
ACTCTTGTTG TCAGACCAGG GAGACCTGAG CATGGCATGT TGAGTTGTAC TCACTCTCCC CAAGACATCT CAGAGAAGTG AACGACCCCA TTAAAGAGGG  4300
CAGCCTGGCC CGGCGCCGTG GCTCACGCCT GTAATCCCAG CACTTTGGGA GCCGAGACA GGCGGATCAC GAGGTCAGGA GATCGAGACC ATCCTGGCTA  4400
ACACGGTGAA ACCCCGTCTC TACTAAAAAT ACCAAAAAAA AAATTAGCGG GGAGCGGTGG CGGGCGCCTG TAATCCCAGC TACTCGGGAG GCTGAGGCAG  4500
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GCGAAATGAG AGTTTAATCT GAGGGTGTGA TCACTTGTCA ACAGAAGTAA TGTACTAATT GGTCCATTCT GGCATTACTA TAAATACCCA AGACTGGGTA  4900
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GATCTCATGA GAAGTCATCG TTAAGGCGAA TCGTGCTAAA CCATTCATGA AAAACCTGCC CTCATGATCC AGTCATCTCC CACCAGGCTC CACCTCCAAC  5200
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TGCTCACAGA TCTCATTCTC CCATCTCCAG GTATACTAAC CATCTATTCT TCTGCCCACA TTTCTTGGTT TACCCAGCCA TCATGCCTCG TGGTCAGAAG  6300
AGTAAGCTCC GTGCCCGTGA GAAACGCCGC AAGCCCCGAG ATGAGACCCG GCGTCTCAAT GTTCCTCAGG TCACTGAAGC AGAGGAAGAA GAGGCCCCCT  6400
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MAGE-B Cluster Sequence

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TGCGGGTGTT	TCATCCACAA	AATCTAAAAA	AGGTGCCAAG	AGCCACCAAG	GTGAGAAAAA	TGCAAGTTCC	TCCCAGGCCT	CAACATCCAC	TAAGAGCCCA	6600
AGCGAAGATC	CTCTAACCAG	GAAGTCAGGG	TCGTTGGTGC	AGTTCCTGTT	GTACAAGTAT	AAAATAAAAA	AGTCCGTTAC	AAAGGGAGAA	ATGCTGAAAA	6700
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CAACGGCCAC	ACTTACACCT	TCATCGACAA	GGTAGACCTC	ACTGATGAGG	AATCCCTGCT	CAGTTCCTGG	GACTTTCCCA	GGAGAAAGCT	TCTGATGCCT	6900
CTCCTGGGTG	TGATCTTCTT	AAATGGCAAC	TCAGCTACTG	AGGAAGAGAT	CTGGGAATTC	CTGAATATGT	TGGGAGTCTA	TGATGGAGAG	GAGCACTCAG	7000
TCTTTGGGGA	ACCCTGGAAG	CTCATCACCA	AAGATCTGGT	GCAGGAAAAA	TATCTGGAGT	ACAAGCAGGT	GCCCAGCAGT	GATCCCCCAC	GCTTTCAATT	7100
CCTGTGGGGT	CCGAGAGCCT	ATGCTGAAAC	CAGCAAGATG	AAAGTCCTGG	AGTTTTTGGC	CAAGGTAAAT	GGTACCACGC	CCCTGTGCCT	TCCCAACCCA	7200
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CAGGGTGGT	GGCGCATGGC	TGTAATGCCA	GCTACTCGGG	AGGCTAAGGC	AGGAGAATCA	CTTGAACCCA	GGAGGCGGAG	GTTGCTGTGA	GCCGAGACTG	8300
CGCCATTGCA	CTCCAGCCTG	GGCAACAAGA	GCGAAACTCC	CTCTAAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAATTGTTGC	ATATTCTCTG	AGATATCCTG	8400
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GGCTGATAGA	AGAAATAGAT	CAATGTATCT	TATCTCTGGC	TGCCCATCTC	CAATCCTAGG	CCCCCTGCTT	TGTGCTACAG	CTTTGCGGTA	TCACACCACT	9100
CCTAGGACAG	AGACCCATTC	TCTGCAATGT	TTACAGAGAG	GAAATTGTTC	TAGAATTTGC	AGAGATGTGG	CATTTTGCAG	GAAGCATTTA	ATCCAGAGAC	9200
AGAGCCAGAT	GAGGGCTGGG	AGACCACATC	CCAGAATATC	AGGCAGGGGT	TTACATAGAC	CATTCCCTTC	TGCTCTCAGA	CAGGGTCCTA	GGCTGAGCTG	9300
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GTCCAGAACC	GTCCTGGTGG	ACACTGACTT	CCAATTCAGG	TCCCAGCAAG	GTGAGGACTT	TGGTCTGAAG	GCTATGGCCT	CAGGTCAGTA	GGGAGTGGAG	9600

MAGE-B Cluster Sequence

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CTCCCAGTTA AGCAAAGAGT GGAATCCCAG ACCATGATAG GTATTAAGGT AAAAATCTCA TATGAGGAAA GAGGCAAACA ACTGCTAGAG AGGTGAACAG  9900
AACAAGGACG TCCTTAGAAA GTTATGCCGG GCCAGACGCG GTGGCTCATG CCTGTAATCC AGCACTTTGG CAGGCCGAGG TGGGCGGATC ATTGAGGTC  10000
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MAGE-B Cluster Sequence

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MAGE-B Cluster Sequence

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MAGE-B Cluster Sequence

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MAGE-B Cluster Sequence

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MAGE-B Cluster Sequence

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MAGE-B Cluster Sequence

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MAGE-B Cluster Sequence

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MAGE-B Cluster Sequence

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TCCCCTGCTG CTGGCATTCC CCAGAAGCCT CAGCGAGCTC CACCCACCAC CACTGCTGCT GCAGCTGTGT CATGTACCGA ATCTGACGAA GGTGCCAAAT 38400
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MAGE-B Cluster Sequence

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CTTGAGGTGA GATGCCAAGA AGCCACTAAG CTAACACTGT TTCCCTAAGC TT                                                    40352
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Claims

1. Method for screening for possibility of a testicular seminoma, non-small cell lung carcinoma, melanoma, breast cancer, sarcoma or leukemia in a sample, comprising contacting said sample with at least one nucleic acid molecule which hybridizes to mRNA corresponding to an MAGE-Xp gene, and determining hybridization as a determination of possible presence of testicular seminoma, non-small cell lung carcinoma, melanoma, breast cancer, sarcoma or leukemia in said sample.

2. The method of claim 1, comprising polymerase chain reaction.

3. The method of claim 2, comprising contacting said sample with at least two nucleic acid primers.

4. The method of claim 1, wherein said MAGE-Xp gene is MAGE-Xp2, MAGE-Xp3 or MAGE-Xp4.

5. The method of claim 4, comprising contacting said sample with (a) SEQ ID NO: 5 and SEQ ID NO: 6, (b) SEQ ID NO: 7 and SEQ ID NO: 8, or (c) SEQ ID NO: 9 and SEQ ID NO: 10.

6. The method of claim 5, comprising contacting said sample with SEQ ID NO: 5 and SEQ ID NO: 6.

7. Isolated nucleic acid molecule consisting of genomic DNA encoding a MAGE-B gene, consisting of nucleotides 3266-7979 of SEQ ID NO:15, nucleotides 23546-25194 of SEQ ID NO:15, 29748-31474 of SEQ ID NO:15, nucleotides 29748-31827 of SEQ ID NO:15, or nucleotides 31403-39691 of SEQ ID NO:15.

8. Isolated nucleic acid molecule which encodes a MAGE-B1 variant, consisting of, in 5′ to 3′ order, nucleotides 31403-31474, 33958-34062, 35057-35139 and 38088-39691 of SEQ ID NO:15; nucleotides 31403-31474, 33958-34062, and 38088-39691 of SEQ ID NO:15; nucleotides 35057-35139 and 38088-39691 of SEQ ID NO:15; and nucleotides 33958-34062 and 38088-39691 of SEQ ID NO:15.

9. Method for determining presence of skin carcinoma

mammary carcinoma, comprising assaying a sample for presence of mRNA for MAGE-B1 gene, wherein presence of said mRNA is indicative of possibility of skin carcinoma or mammary carcinoma in said sample.

10. Method for determining presence of leukemia, lymphoma, head and neck squamous cell carcinoma, or mammary carcinoma in a sample, comprising assaying said sample for mRNA for MAGE-B2, wherein presence of said mRNA is indicative of possibility of leukemia, lymphoma, head and neck squamous cell carcinoma, or mammary carcinoma in said sample.

11. The method of claim 9, comprising determining said mRNA by means of polymerase chain reaction which comprises using one of the oligonucleotides set forth in SEQ ID NO:11, 16 or 17 and the oligonucleotide set forth in SEQ ID NO:12 as primers.

12. The method of claim 10, comprising determining said mRNA by means of a polymerase chain reaction which comprises using the oligonucleotides set forth in SEQ ID NO:5 and SEQ ID NO:6, or SEQ ID NO:18 and SEQ ID NO:6 as primers.

13. Isolated nucleic acid molecule useful as an oligonucleotide primer, said isolated nucleic acid molecule having a nucleotide sequence as set forth in

SEQ ID NO:12,
SEQ ID NO:16,
SEQ ID NO:17, or
SEQ ID NO:18.

14. Kit useful in amplifying a MAGE-B gene, comprising a pair of oligonucleotide primers, said pair being selected from the group consisting of: (a) SEQ ID NO:12 and one of SEQ ID NO:11, 16 or 17, or (b) SEQ ID NO:6 and SEQ ID NO:18.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/09774

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12Q 1/68; C12P 19/34; C07H 21/02, 21/04
US CL :435/6, 91.2; 536/23.1, 24.33
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.2; 536/23.1, 24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	US 5,587,289 A (LURQUIN et al.) 24 December 1996, see especially columns 3, 4, and sequence ID numbers 3, 4, and 5-8.	1-7, 10, 12
A, P	US 5,612,201 A (DE PLAEN et al.) 18 March 1997.	1-14
A	DE BACKER et al. Structure, Chromosomal Location, and Expression Pattern of Three Mouse Genes Homologous to the Human MAGE Genes. Genomics, 1995. Vol. 28. Pages 74-83.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 AUGUST 1997	12 SEP 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer AMY ATZEL, Ph.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/09774

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAPLUS, CANCERLIT, EMBASE, BIOSIS, INPADOC, MEDLINE, NUCLEIC ACID SEQUENCE DATABASES
search terms: MAGE, MAGE-B, MAGE-Xp, testicular seminoma, carcinoma, lung, hybridize, nucleic, skin, mammary, breast, leukemia, lymphoma, head, neck, squamous, polymerase chain, kit, primer